

WHAT IS CLAIMED IS:

1. A process for preparing at least one decolorized heparin product, exclusive of commercially available LMWHs regulated by the USFDA as of the filing date of this application, from heparin comprising:

a) purification of the heparin by oxidation with about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C.; and

b) depolymerization of the oxidized heparin to obtain said at least one decolorized heparin product.

2. The process according to claim 1, wherein the heparin product is purified after depolymerization.

3. The process according to claim 1, wherein the concentration of the at least one permanganate is about 8%.

4. The process according to claim 1, wherein the at least one permanganate is potassium permanganate.

5. The process according to claim 1, wherein the heparin product is a low molecular weight heparin which is not enoxaparin or enoxaparin sodium.

6. The process according to claim 1, wherein the heparin product is an ultralow molecular weight heparin.

7. The process according to claim 1, wherein oxidation occurs at a temperature ranging from approximately 40°C to approximately 80°C.

8. The process according to claim 1, wherein said at least one heparin product is glycoserine-free.

9. The process according to claim 1, wherein said at least one heparin product has a reduced glycoserine content.

10. A method for determining the glycoserine content of a sample of a heparin or a heparin product comprising:

a) treating the sample; and

b) analyzing the sample using a chromatography process to detect the presence or absence of glycoserine and/or oxidized glycoserine residues in the sample.

11. The method according to claim 10, wherein treating the sample comprises depolymerizing the sample.

12. The method according to claim 11, wherein the sample is depolymerized with at least one heparinase.

13. The method according to claim 12, wherein said at least one heparinase is selected from heparinase 1, heparinase 2, and heparinase 3.

14. The method according to claim 11 wherein the sample is depolymerized with a mixture of heparinases comprising heparinase 1, heparinase 2, and heparinase 3.

15. The method according to claim 10, wherein said chromatography process detects acetylated groups.

16. The method according to claim 10, wherein said chromatography process comprises anion-exchange chromatography.

17. The method according to claim 15, wherein said chromatography process comprises strong anion exchange chromatography (SAX).

18. The method according to claim 17, wherein the strong anion exchange chromatography comprises a solid support functionalized with quaternary ammonium exchange groups .

19. The method according to claim 16, wherein the stationary phase is grafted with quaternary ammonium derivatives, which comprise -NMe_3^+ .

20. The method according to claim 10, wherein said chromatography process comprises CTA-SAX chromatography.

21. The method according to claim 16, wherein the mobile phase for anion-exchange chromatography is transparent to ultraviolet (UV) light with wavelengths from about 200 nm to about 400 nm.

22. The method according to claim 21, wherein said mobile phase comprises sodium perchlorate, methanesulfonate salts, or phosphate salts.

23. The method according to claim 10, further comprising a method for detecting polysaccharides following said chromatography process.

24. The method according to claim 23, wherein said polysaccharides are detected by UV absorption.

25. The method according to claim 24, wherein the UV absorption is measured at at least two wavelengths chosen so that the absorption signals of non-acetylated polysaccharides cancel out.

26. The method according to claim 25, wherein the at least two wavelengths include 202 nm and 240 nm.

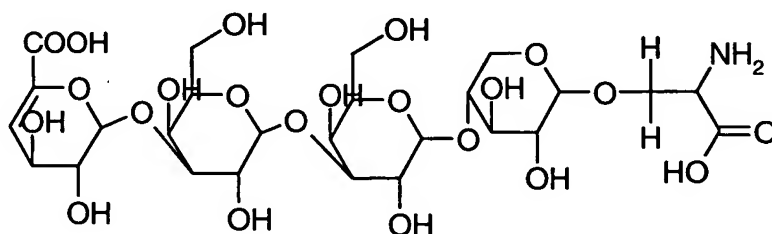
27. The method according to claim 10, wherein the glycoserine content is quantified by external or internal calibration.

28. The method according to claim 27, wherein the internal calibration comprises the use of an internal standard.

29. The method according to claim 28, wherein the internal standard is 2-naphthol-3, 6-disulfonic acid

30. The method of claim 28, wherein the internal standard is about 0.15 g/l of 2-naphthol-3, 6-disulfonic acid.

31. The method of claim 10, wherein said chromatography process comprises detecting a tetrasaccharide of the glycoserine-linking domain of heparin having the following structure:



32. The method of claim 21, wherein the pH of the mobile phase ranges from about 2.0 to about 6.5.

33. The method of claim 32, wherein the pH of the mobile phase is about 3.

34. A method for analyzing a sample of a heparin product in order to detect and/or quantify glycoserine and/or oxidized derivatives of glycoserine in said sample comprising:

- a) depolymerizing the sample;
- b) separating the oligosaccharides in the sample by high pressure liquid chromatography;
- c) detecting and/or quantifying oligosaccharides that contain glycoserine and/or oxidized derivatives of glycoserine.

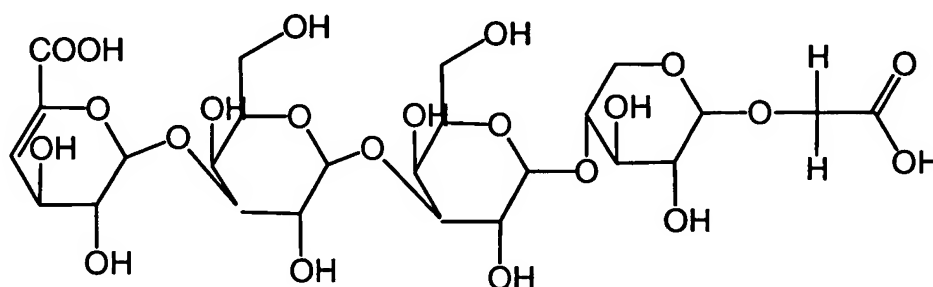
35. The method according to claim 34, wherein the sample is depolymerized through the action of at least one heparinase.

36. The method of claim 35, wherein said at least one heparinase is selected from heparinase 1, heparinase 2, and heparinase 3.

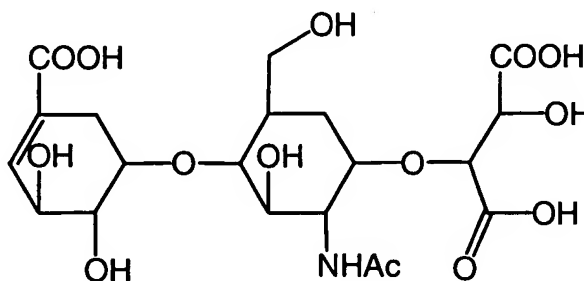
37. The method according to claim 34, wherein said sample is depolymerized through the action of a mixture of heparinases.

38. The method according to claim 37, wherein said mixture of heparinases comprises heparinase 1, heparinase 2, and heparinase 3.

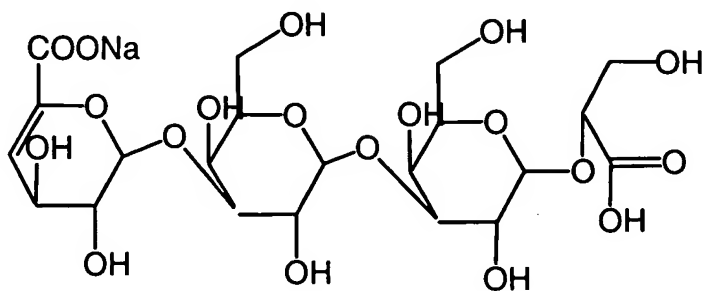
39. A substantially pure compound having the formula:



40. A substantially pure compound having the formula:



41. A substantially pure compound having the formula:



42. A process for preparing at least one decolorized heparin product chosen from fraxiparin, fragmin, innohep (logiparin), normiflo, embollex (sandoparin), fluxum (minidaltan), clivarine, and hibor from heparin comprising:

a) purification of the heparin by oxidation with about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C; and

b) depolymerization by a manufacturer of the oxidized heparin according to a process to obtain said decolorized heparin product.

43. A process for preparing at least one decolorized heparin product chosen from fraxiparin, fragmin, innohep (logiparin), normiflo, embollex (sandoparin), fluxum (minidaltan), clivarine, and hibor comprising: depolymerizing heparin oxidized with about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C, according to a process to obtain said decolorized heparin product.

44. At least one composition chosen from low glycoserine and glycoserine-free, decolorized LMWHs and ULMWHs, exclusive of LMWHs and ULMWHs products approved by the USFDA as of the filing date of this application, obtained according to a process comprising:

a) purification of the heparin by the action of 4 to 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C;

b) depolymerization of the heparin to obtain said at least one composition;
and

c) optionally, purification of said at least one composition.

45. At least one composition chosen from low glycoserine and glycoserine-free, decolorized LMWHs and ULMWHs, exclusive of LMWHs and ULMWHs products approved by the USFDA as of the filing date of this application, obtained according to a process comprising depolymerizing heparin oxidized by the action of 4 to 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C.

46. A process for preparing decolorized enoxaparin from heparin comprising:

a) purification of the heparin by oxidation with about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate,

wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C; and

b) depolymerization by a manufacturer other than one chosen from Aventis Pharma SA, its fully owned subsidiaries, and its successors and assigns, and agents of Aventis Pharma SA, its fully owned subsidiaries, and its successors and assigns, of the oxidized heparin according to a process to obtain said enoxaparin.

47. A process for preparing decolorized enoxaparin comprising:
depolymerization according to a process by a manufacturer other than one chosen from Aventis Pharma SA, its fully owned subsidiaries, and its successors and assigns, and agents of Aventis Pharma SA, its fully owned subsidiaries, and its successors and assigns, of heparin oxidized by about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C, to obtain said enoxaparin.

48. A method for monitoring the glycoserine content of a sample of a heparin or a heparin product comprising:

a) removing and/or diminishing glycoserine and/or oxidized glycoserine residues from the sample; and

b) analyzing the sample using a chromatography process to detect the presence or absence of glycoserine and/or oxidized glycoserine residues in the sample.

49. A method according to claim 48, wherein removing and/or diminishing glycoserine and/or oxidized glycoserine residues comprises

a) purification of the heparin by oxidation with about 4% to about 10% by weight relative to the heparin of at least one permanganate salt chosen from potassium permanganate, sodium permanganate, and quaternary ammonium permanganate, wherein oxidation occurs at a temperature ranging from approximately 35°C to approximately 90°C.; and

b) depolymerization of the oxidized heparin.

50. The method according to claim 49, wherein the heparin or heparin product is purified after depolymerization.

51. The method according to claim 49, wherein the concentration of the at least one permanganate salt is about 8%.

52. The method according to claim 49, wherein the at least one permanganate salt is potassium permanganate.

53. The method according to claim 48, wherein said chromatography process detects acetylated groups.

54. The method according to claim 48, wherein said chromatography process comprises anion-exchange chromatography.

55. The method according to claim 48, wherein said chromatography process comprises strong anionic exchange (SAX).

56. The method according to claim 48, wherein said chromatography process comprises CTA-SAX chromatography.

57. A method of predicting the tendency of a heparin or a heparin product to colorize, comprising measuring the glycoserine content of said heparin or heparin product.

58. The method according to claim 57, wherein the glycoserine content is quantified by external or internal calibration.
59. The method according to claim 58, wherein the internal calibration comprises the use of an internal standard.
60. The method according to claim 59, wherein the internal standard is 2-naphthol-3, 6-disulfonic acid.
61. The method according to claim 59, wherein the internal standard is about 0.15g/l of 2-naphthol-3, 6-disulfonic acid.
62. A method for determining the oligosaccharide content of a sample of a heparin or heparin product comprising:
- a) depolymerizing the sample; and
 - b) analyzing the sample using a chromatography process to detect at least one oligosaccharide chosen from MM=511, MM=588 and MM=690 in the sample.
63. The method according to claim 62, wherein the oligosaccharide content is quantified by external or internal calibration.
64. The method according to claim 63, wherein the internal calibration comprises an internal standard.
65. The method according to claim 64, wherein the internal standard is a substantially pure compound selected from MM=511, MM=588 and MM=690.
66. A method for quantifying the glycoserine content of a sample of a heparin or heparin product comprising enzymatically digesting the sample and quantifying the content of the glycoserine residues in the digested sample by a chromatography method.

67. The method according to claim 66, wherein the chromatography method is HPLC.

68. The method according to claim 66, wherein the chromatography method is CTA-SAX.

69. The method according to claim 66, wherein the chromatography method is SAX.

70. The method according to claim 66, wherein the glycoserine content is reduced below 2% of the sample.